Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Chemistry 8 (2013) 258 – 268

Procedia Chemistry

Youth in the Conservation of Cultural Heritage, YOCOCU 2012

Atomic Force Microscopy as a valuable tool in an innovative multi-scale and multi-technique non-invasive approach to surface cleaning monitoring

C. Pereira[a]*, I.M.P.L.V.O. Ferreira[d], L.C. Branco[c], I.C.A. Sandu[a,c], T. Busani[b]

[a]*Dep. of Conservation and Restoration, Faculty of Science and Technology, New University of Lisbon, 2829-516 Caparica, Portugal*
[b]*CENIMAT, Faculty of Science and Technology, New University of Lisbon, 2829-516 Caparica, Portugal*
[c]*REQUIMTE, Faculty of Science and Technology, New University of Lisbon, 2829-516 Caparica, Portugal*
[d]*REQUIMTE, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal*

Abstract

To monitor and analyze the effectiveness of new cleaning formulations, using a combination of ionic liquids ([BMIM] [BF_4] and [EMIM] [$EtSO_4$]) and enzymes (three different proteases E.C.3.4.), we adopted a novel multi-scale non-invasive approach based with different instruments: the stereomicroscope (SM), the optical microscope (OM) with visible and fluorescence light, atomic force microscope (AFM) and scanning electron microscope (SEM).
The combinations of these techniques allowed an extensive and complete characterization of the surface materials and were successfully applied for monitoring the cleaning process. Although the results showed in this work were obtained for this specific treatment, of removing proteinaceous varnish layer from documented reconstructions, it was demonstrated that the AFM monitoring protocol can be widely applied on everyday situations in the conservation science.

Selection and peer-review under responsibility of the IA-CS (Italian Association of Conservation Scientists) and University of Antwerp

Keywords: AFM, Enzyme, Ionic-liquid, Cleaning, Documented reconstructions

* Corresponding author. Tel.: +351-212-948-300; fax: +351-212-954-461.
E-mail address: catarinacortes@campus.fct.unl.pt.

Selection and peer-review under responsibility of the IA-CS (Italian Association of Conservation Scientists) and University of Antwerp
doi:10.1016/j.proche.2013.03.032

1. Introduction - the project:

The project proposes the novel use of enzymes combined with ionic liquids (IL) for the removal of proteinaceous material from documented reconstructions of painted or gilded surfaces. Here is presented the protocol established for monitoring the cleaning effectiveness.

In the formulations proposed the enzyme is the active cleaning agent and the ionic liquid is an alternate enzyme solvent and carrier of the catalyzed products.

Ionic liquids are called "design solvents" because, by changing cation-anion combinations [1], they can be adjusted to specific situations or requirements, such as biocompatibility, improvement of enzyme cleaning effectiveness and surface compatibility, when common solvents or gels aren't suitable. Due to the low vapor pressure IL are considered potentially "green solvents" [2], this makes them not volatile solvents therefore, easier to control the possible impact on the environment, and safer for the conservator. This is an unstudied area in conservation science with a large number of possibilities.

The ILs were chosen in accordance with relevant literature, for example the works of Moniruzzamana [3], Moon [4] and Park [5]; and availability, and narrow down to two possibilities after pre-cleaning tests done before the actual cleaning trials. These tests were carried out on proteinaceous varnishes prepared as single layer surfaces on glass slides, these results helped also to establish concentrations, cleaning protocol and expected effectiveness.

Documented reconstructions were prepared using traditional painting techniques [6, 7] on wood and canvas with oil and tempera binders mixed with of iron oxides pigments (yellow ochre and red sinopia) and gilding technique [8]. Four proteinaceous varnishes were applied on these surfaces; they were prepared with egg white, isinglass, animal glue and casein respectively, according with traditional recipes [9, 10].

For the removal of the varnish layers, new formulations using a combination of an ionic liquid ([BMIM] [BF_4] or [EMIM] [$EtSO_4$]) and enzymes (three different proteases E.C.3.4. at pH of 5, 7 and 8.4) were applied to the surface following the basic enzyme cleaning protocol as described by Cremonesi [11] or Wolbers [12]: applied at 37^{o}C at different periods of time; according with pre-cleaning tests done for each protein material. After enough time (from 30 seconds to 30 minutes), to allow enzyme catalysis, the formulation is removed mechanically, along with the product of hydrolysis, with a dry cotton swab. The final clearance process, with successive cotton swabs with water and white spirits, was performed to remove all residues of varnish and cleaning material.

Nomenclature

IL	Ionic Liquid
[BMIM] [BF_4]	1-butyl-3- methylimidazolium tetrafluoroborate
[EMIM] [$EtSO_4$]	1-ethyl-3-methylimidazolium ethyl sulfate
E.C.3.4.	Enzyme Classification, Hydrolases acting on peptide bonds
SM	Stereomicroscope
OM	Optical Microscopy
AFM	Atomic Force Microscopy
SEM	Scanning Electron Microscopy

1.1. Establishing the analytical protocol.

To monitor and assess effectiveness of the treatment an analytical protocol was established and used before and after the removal of the protein-based varnish. This protocol is based on the complementary use of different microscopy techniques, non invasive and with little or no sample preparation. The comparison of the images before and after treatment of the same surface allowed us to characterize the surface, but also, gather information about cleaning effectiveness, possible residues, and other features in useful time.

This is a multi-scale protocol with some of the more common techniques from the stereomicroscope (SM) at 10x magnifications, then the optical microscope (OM) and scanning electron microscope (SEM) on uncoated samples allowing good resolution imaging up to 3000x magnification. The atomic force microscopy (AFM) was also introduced, a topographic imaging technique, at an image size of 5 microns. For the AFM other images were also obtained, aside for the height variation/topographic image, namely the amplitude image which registered the tip changes, and phase image, for the tip interaction with the surface.

The atomic force microscopy is a nano-scale technique designed to measure the topography of a non conductive sample. Some work has been done with this technique for cultural heritage purpose; to name only two, relevant works one of Doménech-Carbó [14] which makes reference to the use of AFM as a tool for material characterization, and other of de Sá on ageing and cleaning effects on the surface of cultural objects [15]. The purpose of the present paper is to show the usefulness of the systematic use of the AFM when studying cultural objects surfaces; and establish it as powerful complementary technique to other more conventional imaging techniques [16].

The basic AFM measurements are qualitative as they render three dimensional images of the scanned surfaces allowing us to have a clear view of its topography. But AFM also can offer some scale and spatial metrology of the surface features at a nano scale.

1.2. Instrumental assets.

An Olympus stereomicroscope system SZX12, mounted on an extendable arm SZ-STU2 with a digital camera DP-12 and an independent light source HighLight 3100 was used to record the sample areas at 10x, 32x and 90x magnifications. Then, Axioplan Zeiss 2 imaging binocular microscope (at 50x, 100x and 200x), with both visible (dark field) and fluorescent radiation, coupled to a Nikon DXM1200F digital camera was used to image the surfaces, before and after the removal of the varnish layers. The blocks of filters used for observing the fluorescence were: BP 300-400, FT 395 and LP 420 (filter 8); BP 450-490, FT 510 and LP 515 (filter 6).

Scanning Electron Microscopy: An Auriga CrossBeam Workstation (SEM-FIB) was used without coating of the sample, operating at 1-5 kV, with a typical working distance (WD) 4.5-9.6 mm, aperture size 30Rm, magnifications: from 200x up to 3000x. Coupled an oxford EDS instrument detector x-act sensor 51-1385-016 with high resolution (at 5KeV).

Atomic Force Microscopy: A MFP-3D Asylum instrument was used at non-contact imaging mode (AC mode) using Si probes Tap300Al from Budget Sensors. The scan size was 5 x 5 μm^2 and the resolution 512 by 512 lines.

The images are shown in a gray scale, where brighter areas represent higher areas. Measurements of surface roughness (R), as the average value of three scanned areas, were obtained with Igor Pro 6.2 Software.

2. Results and discussion:

For each sample a set of images was acquired, this gave us a surface characterization for each paint/gilding material and artistic technique. Although repeated patterns were observed these results are to be understood as

patterns of these samples and not standards of a specific artistic technique. This is because the surface pattern varies according with the material used, the application method, type of brush, number of layers; and for some materials even the temperature at the time of application can be an important factor, but also the material and shape of the layers beneath. Unvarnished samples were prepared to be used as reference of after cleaning surfaces.

2.1. Surface characterization – Qualitative information.

Figure 1 shows an example of a set of images obtained by various microscopic techniques for a sample of tempera painting with isinglass varnish, in this case, before the cleaning treatment.

From this set of images we can extensively characterize this tempera painting surface. The SM registers the surface close to the human eye perception (figures 1a, b), this is important especially for documentation purposes of the surface before treatment. Both SM and OM characterized the surface in terms of color (hue and tone – figures 1a-c). The OM goes further registering the general appearance of the varnish layer, aspects such as, glossiness and transparency or smoothness; also size and color of the pigment grains. Surface defects, such as gaps or fissures, can become apparent at this scale. Some OM images were obtained with an ultraviolet (UV) light source which added information when fluorescence occurred. Here as an overall blue appearance (figure 1d) characteristic of the protein material of the isinglass varnish [16].

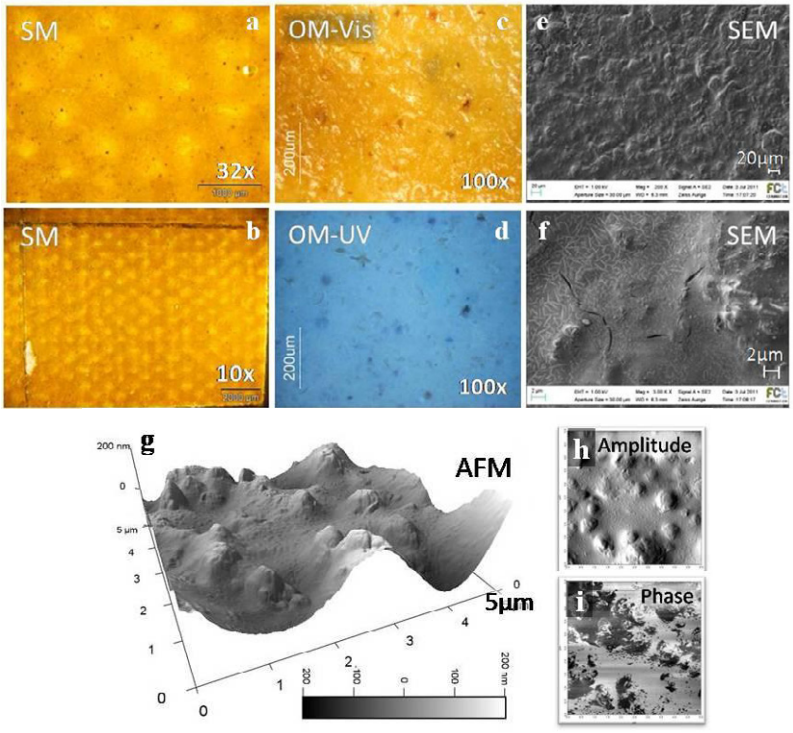

Fig. 1. Multiscale images of a tempera painting sample with isinglass varnish, obtained by SM (a, b); OM with visible light (c) and UV light (d); SEM (e, f) and 2D and 3D rendering of the AFM image (g-i).

In this case, SEM and AFM, figures 1e,f and figures 1g-i respectively, gave similar information of the granular aspect of the surface with the advantage of AFM being able to measure at atmosphere pressure, which

allows scanning more fragile surfaces. The granular aspect of these images demonstrates that the varnish layer does not cover the surface in a uniform manner. The varnish fills the deepest areas leaving almost uncovered the most prominent features. The Phase image shows that the interaction of the tip does not follow completely the topography indicating differences or in the material present or in the molecular arrangement of the same material (more or less crystalline, here possibly related to the layer thickness).

2.2. Surface characterization – Quantitative information.

At least three AFM images were obtained for each sample in order to have statistical probability of the surface features. Roughness measurements (R) were done for each surface as we can see in Table 1.

Table. 1. Roughness values of the studied samples and reference (unvarnished) samples.

Standard unvarnished surfaces		Surface roughness (R)	Dispersion (%)
Canvas	Tempera	59.42	2.76
	Oil	61.20	14.49
Wood	Oil	73.91	7.55
	Gold Gilding	11.31	2.26

Varnished surfaces		
Tempera + Egg White	6.57	3.04
Tempera + Isinglass	39.93	6.06
Oil + Egg White	2.37	9.05
Oil + Isinglass	34.84	7.08
Oil + Casein	4.54	6.59
Gold Gilding + Animal Glue	14.68	0.83

The comparison with the reference unvarnished samples allows to establish the alteration introduced by the varnish layer on the surface. For example, all the surfaces except the gilded ones, show lower roughness values after varnishing. This can be easily related with the change in the optical properties, as the varnish smoothes the surface, changing the refractive index and increasing specular reflection.

The gilded surfaces are the exception, and this is related with the purpose of the varnish. The animal glue is applied in order to lower the glossiness, which can be corroborated with the AFM measurements, showing in turn an increase on the surface roughness (table 1).

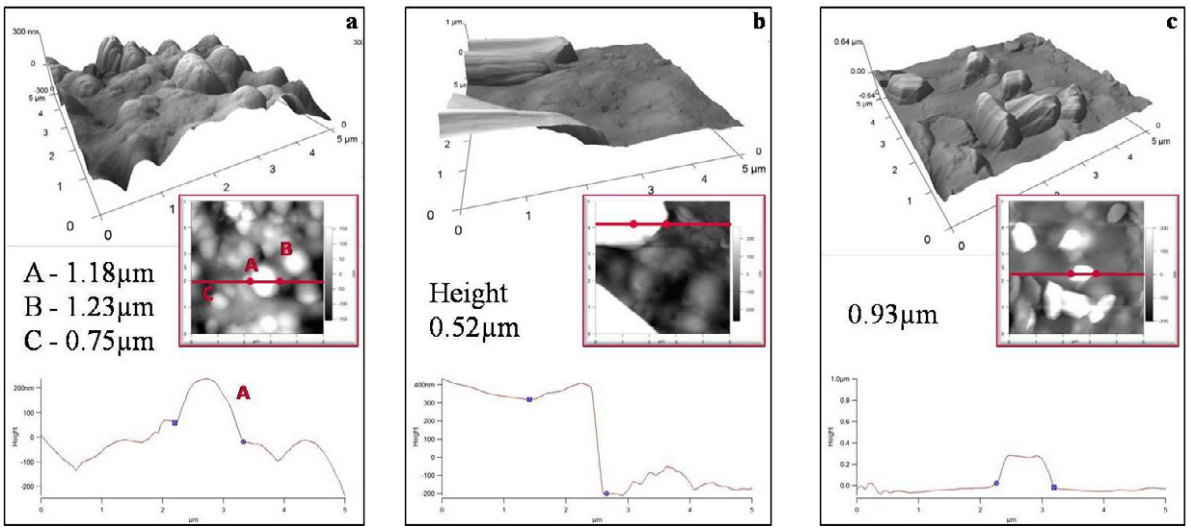

Fig. 2. 2D and 3D rendering of the AFM topographic image and the profile graphic obtained at the level showed by the red lines respectively. (a) Measurements of particle size of pigments on an egg tempera unvarnished sample; (b) Layer height of a egg white varnish measured on a gap area; (c) Measurements of crystals size of foreign material present on the surface after cleaning.

It is also possible to obtain profile graphics from the AFM images (figure 2). These are useful for measuring surface features length such as particle size of pigments (figure 2a). In figure 2b we can measure the height of the varnish layer in a gap area, and some information can be gathered in relation to the presence of residues as showed in figure 2c, the polyedric structures observed suggests the formation of crystals on the surface.

2.3. Cleaning effectiveness assessment.

Cleaning effectiveness assessment was done by cross-correlation of each analytical technique used in this study, by comparing the before treatment with the after treatment images and also with the images of reference unvarnished samples.

The interpretation of data for the successful cleaning is simple and immediate: images after treatment should be similar to the reference unvarnished samples. Figure 3a shows a tempera painting sample after treatment with

[EMIM][$EtSO_4$] and the alkaline enzyme for the removal of egg white varnish. In this case the three AFM images helped to establish that this as a successful cleaning as the surface features repeat themselves and are similar to the unvarnished reference sample (figure 3b).

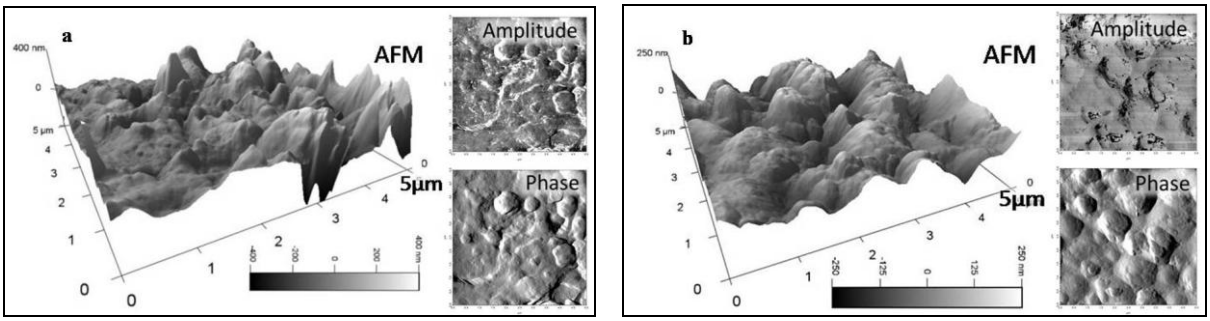

Fig. 3. 2D and 3D rendering of the AFM image set of topography, amplitude and phase (a) Tempera painting sample after cleaning; (b) Unvarnished reference sample of tempera painting.

Figure 4 shows the surface of a gilded sample before and after treatment for the removal of animal glue coating. The OM image obtained under UV light source show total loss in fluorescence after treatment, ascertaining the absence of proteinaceous material (figure 4b), as the gold leaf has no fluorescence under UV light. Therefore it can be assessed that the cleaning treatment was effective, figure 4a. However, the topographic image obtained with AFM shows surface degradation and the presence of grooves; suggesting the effect of scratching left by the cotton swabs, maybe because of excessive pressure when cleaning.

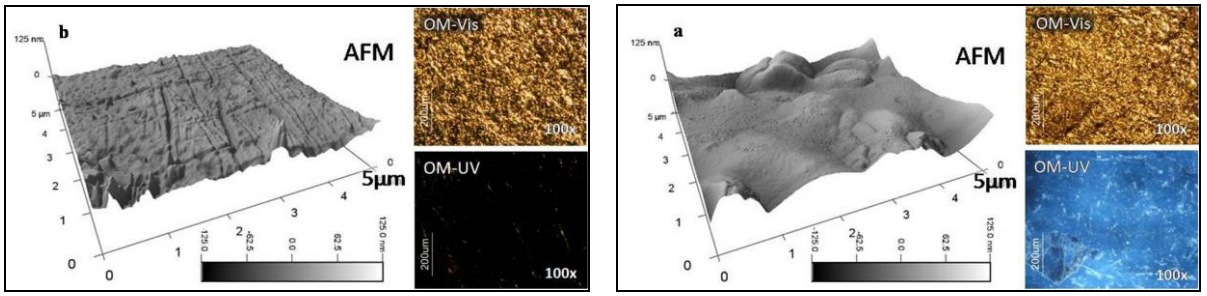

Fig. 4. 3D rendering of the AFM topographic image and OM with visible light and UV light, images set of animal glue coating from a gilded surface sample (a) Before cleaning (b) After cleaning.

2.4. Assessing residues – Varnish.

When assessing effectiveness of the cleaning protocol, one of the important parameters to be studied was the presence or absence of varnish residues after cleaning.

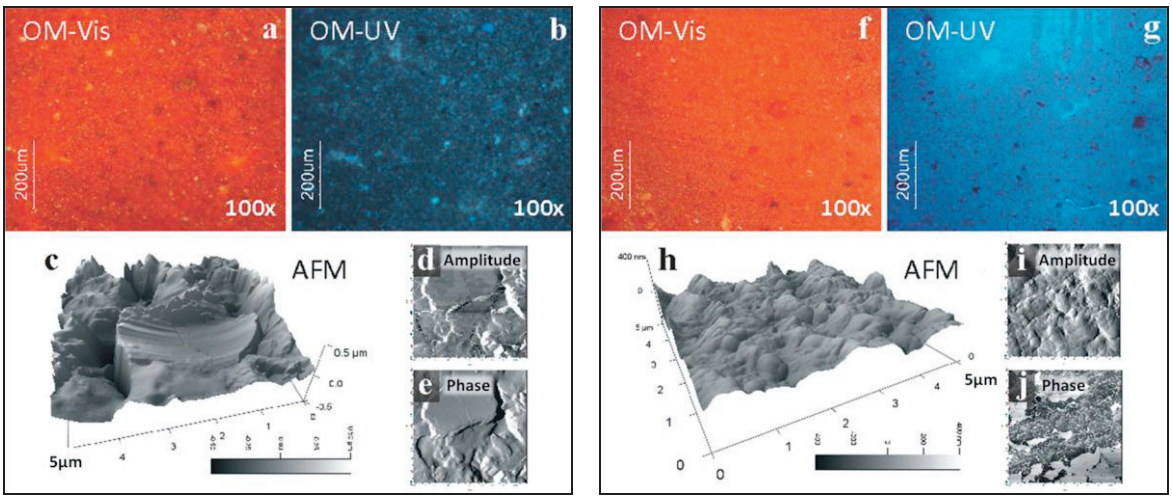

Fig. 5. 2D and 3D rendering of the AFM images and OM with visible light and UV light, images set after removal of isinglass varnish (a-e) Oil painting sample (f-j) Tempera painting sample.

The figure 5 shows two samples cleaned with the same formulation: the alkaline enzyme with IL [BMIM][BF_4] for the removal of isinglass varnish; second sample, figure 5f-j of a tempera painting and second sample figure 5a-e of an oil painting. The presence of varnish residues was detected by the different imaging techniques used. The image obtained with the OM, under UV for the second sample (figure 5d), made possible to registered protein residue as it gives a different fluorescence effect on the surface. But the same was not observed on the correspondent image of the other sample in figure 5b. But, at a smaller scale, for both samples, AFM registered the residue as flat plates, in different sizes, between crests. In the AFM phase image (figure 5g), a different interaction of the tip with the surface is observed resulting in areas with higher contrast. The white areas are similar in texture to the surrounding material: an explanation can be that, there is a change in the nature of the material present. Correlating with the other images it is possible to conclude that in fact the white areas are varnish residue.

2.5. Assessing residues – Ionic liquid.

Another important aspect in evaluating the cleaning effectiveness is the ionic liquid residue. A small set of the tempera painting samples cleaned with [BMIM][BF_4] shows polyhedric crystals on the surface, visualized with both SEM and AFM techniques (figure 6a, b). These structures were not found before cleaning in any of the studied samples; therefore the presence of these structures is the result of the cleaning treatment, as residues of material left during treatment, most probably residues of the IL. All the materials used on the samples are of organic nature therefore little compositional information can be obtained with SEM. But, for the [BMIM] [BF_4] the Fluorine from the anion can be detected by Energy-dispersive X-ray spectroscopy with the low intensities used (F kα at 677eV) with the uncoated samples Figure 6a shows the elemental mapping of Fluorine on the surface as green spots, identifying the observed crystal as being in fact IL crystals. Because this only happened in

a set of 4 samples, all done at the same time, and because this was not observed on other samples where the same IL was used, we concluded that this was a flaw in the cleaning protocol and attention should be paid especially to the clearance steps.

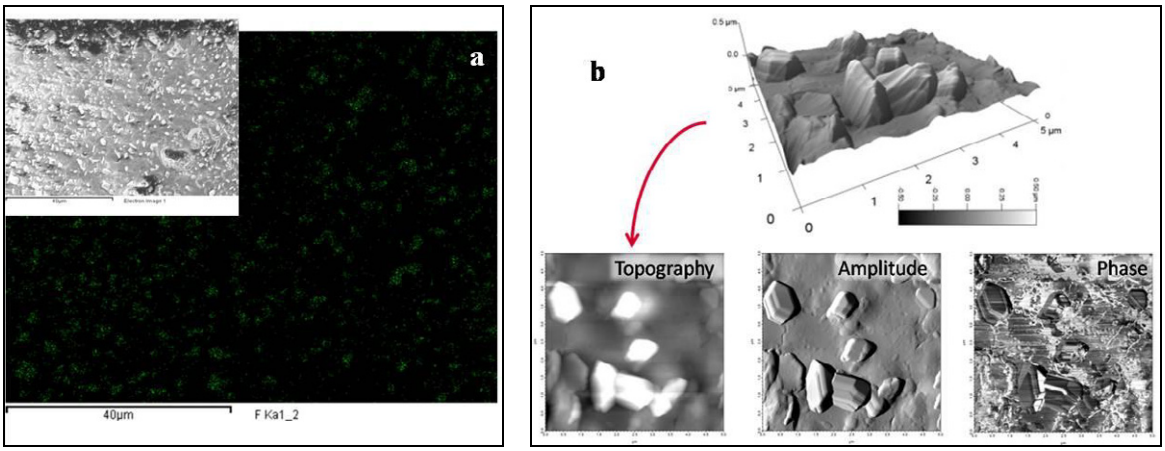

Fig. 6. Tempera painting sample after removal of egg white varnish with cleaning formulation: [BMIM][BF_4] and acidic enzyme (a) Elemental mapping with SEM, (b) 2D and 3D rendering of the AFM image set.

3. Conclusions:

The AFM used in cross-correlation with the other imaging microscopic techniques is useful for surface characterization, assessing treatment effectiveness and presence of residues in a qualitative and quantitative approach.

- The information retrieved with the different techniques allowed a multi-scale surface characterization of documented reconstructions of painted and gilded composites of cultural heritage objects.
- Not only qualitative information was obtained by AFM but also quantitative, when measuring surface roughness and sections profiles using specific software.
- Going to a nano-scale with the use of AFM proved to be of major importance to achieve a further level of information on the studied surfaces; at the same time the conclusions were easily extrapolated to a larger scale.
- An important advantage of the analytical techniques used resides in the real time monitoring of the cleaning process, which allows information available for possible necessary adjustments.

4. Further studies:

AFM can be further applied as a routine analytical tool in conservation studies. This will allow the construction of databases of scientific data from real and reference samples of different materials and techniques, for better understanding patterns and behaviors of artistic surfaces in the conservation and restoration practice.

Acknowledgements

The authors would like to acknowledge the Fundação para a Ciência e a Tecnologia for financial support through grant no. PEst-C/EQB/LA0006/2011; and the 7th Framework Programme of the EU (CHARISMA Grant Agreement n. 228330) for the financial support and access to research infrastructures.

References

[1] Branco LC, Carrera GVSM, Aires-de-Sousa J. Physico-chemical properties of task-specific ionic liquids. In: Kokorin A, editor. *Ionic liquids: theory, properties, new approaches*. Croatia: InTech; 2011, p. 61–94.

[2] Tan Z, Welz-Biermann U, Yan P. Thermodynamic properties of ionic liquids - Measurements and predictions. In: Kokorin A, editor. *Ionic liquids: theory, properties, new approaches*. Croatia: InTech; 2011, p. 3–36.

[3] Moniruzzaman M, Nakashima K, Kamiya N, Goto M. Recent advances of enzymatic reactions in ionic liquids. *Biochemical Engineering Journal*. Elsevier, 2010; **48**: 295–314.

[4] Moon Y, Lee SM, Ho Ha S, Koo Y. Enzyme-catalyzed reactions in ionic liquids. *Korean J. Chem*. 2006; **23-2**: 247–263.

[5] Park S, Kazlauskas R. Biocatalysis in ionic liquids - Advantages beyond green technology. *Current Opinion in Biotechnolog*y. Elsevier 2003; **14**:432–437.

[6] Carlyle L. *The Artist's Assistant. Oil painting Instruction Manuals and Handbooks in Britain 1800-1900 with reference to selected eighteenth-century sources.* London: Archetype Publications; 2001

[7] Cennini C. *Il libro dell'arte o trattato della pittura / di Cennino Cennini; a cura di Fernando Tempesti*. Milano: Biblioteca Longanesi; 1984.

[8] Sandu ICA, Afonso LU, Murta E, de Sá MA. Gilding Techniques in Religious Art Between East and West, 14th -18th Centuries. *International Journal of Conservation Science* 2010; **1-1**:47–62.

[9] Gettens R, Stout G. *Painting Materials: A Short Encyclopaedia.* New York: Dover Publications; 1966.

[10] Westhoff H. Coatings of medieval gilded surfaces and their deterioration. In *Gilded wood: conservation and history: Postprints of the Gilding Conservation Symposium. 19*, Madison, Connecticu: Philadelphia Museum of Art; 1988, p. 79–86.

[11] Cremonesi P. *L'uso degli enzimi nella pulitura di opera policrome.* Padova: Il Prato Editore; 2001.

[12] Wolbers RC. *Cleaning Painted Surfaces, Aqueous Methods*, London:Archetype Publications; 2000.
[13] Doménech-Carbó MT. Novel analytical methods for characterising binding media and protective coatings in artworks. *Analytica Chimica Acta* 2008; **621**:109–139.
[14] de Sá M H, Ferreira JL, Melo M J, Ramos A M. An AFM contribution to the understanding of surface effects caused by ageing and cleaning on acrylic glass. The Shadows by Lourdes Castro, a case study. *Surf. Interface Anal* 2010; **43**: 1165–1170.
[15] de la Rie ER. Fluorescence of paint and varnish layers (Part III). *Studies in Conservation* 1982; **27-3**: 102–108.
[16] Sandu ICA, Pereira MC, de Sá MH. Ancient "gilded" art-objects from the European cultural heritage: a review on different scales of characterization. *Surf. Interface* Anal 2011; **43**: 1134–1151.